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Inhibition of a Langerhans Cell-Mediated Immune Response by Treatment Modalities Useful in Psoriasis*

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Neither the pathogenesis of psoriasis nor the mechanism whereby seemingly diverse therapies alter the disease is understood. In this study, several antipsoriatic agents were tested for their effects on the skin cell lymphocyte reaction (SLR), an immunologic assay in which HLA-DR antigens on Langerhans cells (LC) stimulate proliferation of allogeneic lymphocytes. Every agent tested (cortisol, methotrexate, hyperthermia, anthralin) inhibited the SLR at therapeutic dose levels. By contrast, a variety of antibiotics, an anti-inflammatory agent, and lithium carbonate and propranolol, two drugs known to be ineffective in psoriasis, failed to inhibit the

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Abbreviations:
Con-A: concanavalin A
EB: ethidium bromide
FCS: fetal calf serum
FDA: fluorescein diacetate
[^{3}H]dThd: tritiated thymidine
HLA-DR antigen: the human equivalent of the murine Ia or immune response-associated antigen
LC: Langerhans cells
8-MOP: 8-methoxypsoralen
MTX: methotrexate
PBML: peripheral blood mononuclear leukocytes
PBS: phosphate-buffered saline
PUVA: photoactivated 8-methoxypsoralen
SLR: skin cell lymphocyte reaction

SLR. Finally, we have shown that hyperthermia and anthralin treatments are toxic for LC whereas they have little or no effect on keratinocyte viability. These results suggest that antipsoriatic agents may act in psoriasis by alteration or killing of LC.

Psoriasis is a disease of unknown etiology characterized by hyperproliferation of keratinocytes [1]. Photochemotherapy with 8-methoxypsoralen (8-MOP) and long-wavelength ultraviolet radiation (360 nm), termed PUVA, usually results in clearing of psoriasis [2]. In this regard, it is interesting that PUVA is effective in a number of skin diseases thought to have an immunologic basis (e.g., vitiligo, alopecia areata) [3,4]. We recently reported that the in vitro treatment of skin cells with PUVA inhibits the capacity of these cells to stimulate allogeneic lymphocytes in the skin cell lymphocyte reaction (SLR) [5]. In the current study, we tested other antipsoriatic treatments for their effects on the SLR, which represents the proliferative response of T-lymphocytes to HLA-DR antigen expressed on epidermal Langerhans cells (LC) [6,7]. The results indicate that every antipsoriatic agent tested is a potent inhibitor of the SLR whereas a variety of antibiotics, anti-inflammatory agents, and other drugs known to be ineffective in psoriasis have no inhibitory effect. The possibility that inhibition of this immune response is related to therapeutic efficacy in psoriasis is discussed.

MATERIALS AND METHODS

Preparation of Skin Cells

Single cell suspensions of skin cells, consisting mainly of epidermal cells, were prepared from normal skin obtained at surgery according to the method of Liu and Karasek [8]. Briefly, trimmed skin was cut into 1 × 5 cm strips and split-cut with a Castroviejo keratotome set at 0.1 mm prior to treatment for 30 min at 37°C with 0.3% trypsin (Microbiological Assoc., Walkersville, Maryland) in 0.8% NaCl, 0.04% KCl, 0.1% glucose, .084% $NaHCO_3$, at pH 7.3, plus 0.1% EDTA. The resultant dispersed cells were then suspended in complete RPMI medium (RPMI 1640, Microbiological Assoc.) supplemented with 10% decomplemented pooled human serum, 2 mM L-glutamine, 100 units/ml penicillin, 50 μg/ml gentamicin, and 100 μg/ml streptomycin) at a concentration of 5×10^5 cells/ml.

"Panning"

A 10 × 2.5 cm Lab Tek Petri dish (Scientific Products) was coated with goat antimouse IgG (Tago, Inc.) at 10 μg/ml in 0.05 M Tris buffer, pH 9.5 [9]. The plates were washed 3 times with phosphate-buffered saline (PBS) and once with 5% fetal calf serum (FCS) in PBS. Dispersed epidermal cells (30×10^6) were incubated with 1 ml OKT6 (diluted 1:50 in PBS) for 20 min at room temperature. The cells were washed twice with PBS, resuspended in 5% FCS/PBS (5×10^6 cells/ml), and 3 ml of this cell suspension was layered onto 1 Petri dish surface, incubated at 4°C for 40 min, swirled, and incubated for another 30 min [9]. The supernatant containing unattached cells was poured off gently, the Petri dish surface washed 5 times with 1% FCS/PBS, and the attached cells were scraped off with a rubber policeman.

Skin Cell-Lymphocyte Reaction (SLR)

Viable epidermal cells (5×10^4) or LC-enriched or LC-depleted epidermal cell fractions were coincubated with 5×10^4 allogeneic peripheral blood mononuclear leukocytes (PBML) in complete RPMI medium in microtiter wells at 37°C in an atmosphere of 5% CO_2/95% air [10]. On the sixth day of culture, 1 μCi of [^{3}H]thymidine ([^{3}H]dThd) (New England Nuclear, Boston, Massachusetts) was added per well, and 18 h later the cultures were harvested with a Mash II apparatus (Microbiological Assoc., Bethesda, Maryland). The incorporation of [^{3}H]dThd was measured in a liquid scintillation counter (Beckman, Irvine, California).

Stimulation of PBML with Concanavalin A

One hundred thousand PBML were incubated in 0.2 ml of complete RPMI in microtiter wells. At the time of incubation, all of the cells received 50 μg/ml concanavalin A (Con-A) to stimulate DNA synthesis. At the same time, some cells were treated additionally with various concentrations of cortisol. Three days later, [^{3}H]dThd (1 μCi/well) was added and 18 h later the cells were harvested with a Mash II apparatus.

Assessment of Cell Viability

At various time intervals, the viability of skin cells was assessed either by trypan blue exclusion or with a sensitive fluorochromatic assay [11]. Briefly, 1.0 ml of cell suspension was incubated with 0.1 ml of 0.01% fluorescein diacetate (FDA) for 5 min in the dark, washed with PBS, and resuspended at 2×10^6 cells/ml in complete keratinocyte growth medium. One drop of this suspension was placed on a slide with 5 μl of ethidium bromide (EB) (0.04% in PBS) and examined immediately with a fluorescence microscope. Live cells show green fluorescence whereas dead cells stain red.

Anthralin Treatment

One milligram of anthralin powder (K&K Labs, Plainview, New York) was dissolved in 1 ml acetone, and 9 ml of complete growth medium was added to achieve a stock concentration of 100 μg/ml. Prior to use, this solution was exposed to air for at least 20 min to allow evaporation of the acetone. Cells were incubated at 37°C for 2 h with anthralin at concentrations up to 10 μg/ml, washed once with 5 ml of complete growth medium, and resuspended in complete RPMI prior to addition to the SLR. Viability was determined at 24 and 48 h. Since anthralin treatment results in brown discoloration of cells which might cause quenching in the scintillation counter, a quench curve was obtained. At the anthralin concentrations used, no quenching resulted.

Hyperthermia Treatment

One million dispersed skin cells in complete growth medium were aliquoted into 6-cm Petri dishes (Lux, M. A. Bioproducts, Walkersville, Maryland) and placed in a water bath controlled to ±1°C and heated to 43°C for up to 4.5 h. After treatment, the cells were removed from the plates and resuspended in complete RPMI prior to addition to the SLR. Viability was determined at 1, 24, and 48 h.

Hydrocortisone, Epicortisol, and Methotrexate (MTX) Treatment

Dispersed cells were incubated with various concentrations of hydrocortisone and epicortisol, ranging from 10^{-5} M to 10^{-8} M, for 24 h in complete growth medium. In a separate experiment, skin cells were incubated in up to 50 μg/ml MTX. The cells were washed twice in complete growth medium and resuspended in complete RPMI medium prior to addition to the SLR. Viability was determined at 24 and 48 h. In another series of experiments, cortisol and MTX were maintained in the culture medium for the duration of the SLR.

Treatment with Other Agents

A variety of "control" agents not known to be efficacious in psoriasis were tested for their effects on the SLR by adding various concentra-

TABLE I. *Effect of pretreatment of skin cells with anthralin on the SLR*

Anthralin pretreatment (2 h)	Response of allogeneic lymphocytes (cpm)[a]			
	Donor A	Donor B	Donor C	Donor D
None[b]	43,991 ± 4,020[c]	31,327 ± 3,033	41,047 ± 4,024	26,400 ± 2,858
0.1 μg/ml	30,273 ± 3,512	40,663 ± 4,038	35,529 ± 3,450	39,891 ± 4,289
0.5 μg/ml	34,860 ± 3,641	43,765 ± 4,417	33,792 ± 3,214	24,856 ± 2,582
2.5 μg/ml	12,648 ± 1,105	2,688 ± 2,556	508 ± 63	17,167 ± 1,760
10.0 μg/ml	101 ± 15	298 ± 25	798 ± 71	103 ± 19

[a] Responder lymphocytes incubated alone incorporated less than 1000 cpm.
[b] Control cells were treated with the same concentration of acetone (1%) as those cells which received the highest anthralin concentration.
[c] Mean of triplicate wells ± SEM.

tions of drugs to skin cell suspensions 2 h before addition of allogeneic lymphocytes and maintaining the cells in the drug for the duration of the SLR. Drugs tested included gentamicin, penicillin, streptomycin, indomethacin, lithium carbonate (Li_2CO_3), and propranolol.

RESULTS

Effect of Anthralin on the SLR

Pretreatment of skin cells with anthralin resulted in dose-dependent inhibition of the SLR. As shown in Table I, preincubation with 0.5 μg/ml or less anthralin failed to inhibit the SLR, whereas 10 μg/ml completely blocked the reaction. To rule out the possibility that the inhibition of the SLR was due to an effect on responder cells of anthralin eluted from stimulator cells during the 7-day incubation, we added 2.5×10^4 anthralin-pretreated (10 μg/ml for 2 h) epidermal cells to 2.5×10^4 untreated epidermal cells and incubated them with 5.0×10^4 allogeneic responder lymphocytes (Table II). The capacity of untreated skin cells coincubated with anthralin-pretreated skin cells to stimulate allogeneic lymphocytes was the same as untreated skin cells coincubated without anthralin-pretreated cells. Thus, anthralin is not leached out of stimulator cells during the 7-day coincubation with allogeneic lymphocytes; the inhibition seen in the SLR by anthralin pretreatment of skin cells must be due to an effect on the stimulator cells alone.

TABLE II. *Effect of anthralin-pretreated skin cells on the capacity of untreated skin cells to stimulate allogeneic PBML*

Pretreatment[a] (10 μg/ml anthralin) for 2 h)	Response of allogeneic lymphocytes (cpm)[b]	
	Donor A	Donor B
None (2.5×10^4 cells)	45,813 ± 3,779[c]	22,488 ± 931
Anthralin (2.5×10^4 cells) plus untreated cells (2.5×10^4)	52,056 ± 4,473	22,339 ± 2,651

[a] In this experiment anthralin pretreatment (10 μg/ml for 2 h) of stimulator cells resulted in over 50% inhibition of the SLR.
[b] Responder lymphocytes incubated alone incorporated less than 1000 cpm.
[c] Mean of triplicate wells ± SEM.

TABLE III. *Effect of pretreatment of skin cells with hyperthermia on the SLR*

Heat (43°C) pretreatment (h)	Inhibition of response of allogeneic lymphocytes (% control)					
	Exp I		Exp II		Exp III	Exp IV
	Donor 1	Donor 2	Donor 3	Donor 4	Donor 5	Donor 6
1	43.2	53.8[a]			27.7[a]	54.9[a]
1.5			27.9[a]	54.8		35.5[a]
2	1.6[a]	25.9[a]			3.3[a]	
2.5			4.1[a]	27.4[a]		3.0[a]
3	1.0[a]	2.1[a]			2.0[a]	
4.5	1.9[a]	0.4[a]			4.0[a]	

[a] Statistically significant inhibition ($p < .05$).

Effect of Hyperthermia on the SLR

As shown in Table III, preincubation of skin cells at 43°C for 2 h or longer significantly inhibited the capacity of these cells to stimulate in the SLR. Similar results were obtained in 4 separate experiments using PBML from 6 unrelated individuals as responder cells.

Effect of Cortisol on In Vitro Immune Responses

Skin cells were preincubated with concentrations of cortisol ranging from 10^{-8} M to 10^{-5} M for 24 h before coincubation with allogeneic lymphocytes in the SLR. None of the concentrations tested resulted in inhibition of the capacity of skin cells to stimulate in the SLR (not shown). On the other hand, addition of the same concentrations of cortisol to the incubation medium at the time of coincubation of skin cells and PBML and maintenance in the medium for 7 days resulted in dose-dependent inhibition of the SLR (Table IV). As a control for these experiments, we used epicortisol, an isomer of cortisol known as an inactive steroid [12]. As high as 10^{-5} M epicortisol in the 7-day incubation medium did not result in inhibition of the SLR (Table IV).

To determine whether the effect of cortisol is only on the stimulator cell population or whether all or part of the effect is on the responder cell population, PBML were incubated with Con-A plus varying concentrations of cortisol (Table V). Cortisol concentrations above 10^{-7} M decreased the Con A-induced stimulation of PBML although the effect after the 4 days of incubation used for this assay is not as strong as that seen after 7 days of incubation in the SLR (Table IV).

Effect of MTX on the SLR

Preincubation of skin cells for 24 h with MTX at concentrations as high as 50 μg/ml did not affect the capacity of these cells to stimulate allogeneic lymphocytes (not shown). By contrast, addition of as little as 0.2 μg/ml to the incubation medium

TABLE V. *Effect of cortisol on Con-A stimulation of PBML*

Stimulus	Response of PBML (cpm)	
	Donor A	Donor B
Con-A only[a]	60,827 ± 8,968[b]	77,426 ± 7,257
10^{-8} M Cortisol + Con-A	60,737 ± 1,253	79,493 ± 11,107
10^{-7} M Cortisol + Con-A	48,510 ± 5,440	87,799 ± 2,678
10^{-6} M Cortisol + Con-A	24,636 ± 3,190	36,625 ± 499
10^{-5} M Cortisol + Con-A	22,973 ± 152	38,553 ± 3,163

[a] All cultures were incubated with 50 μg/ml Con-A. All drugs were added only once—at the time of lectin addition to PBML.
[b] Mean of triplicate wells ± SEM.

TABLE IV. *Effect of cortisol or epicortisol on the SLR*

Treatment[a]	Response of allogeneic lymphocytes (cpm)			
	Donor A	Donor B	Donor C	Donor D
None[b]	48,651 ± 3,634[c]	22,707 ± 3,105	56,355 ± 8,515	22,160 ± 2,839
10^{-8} M Cortisol	34,379 ± 2,871	22,871 ± 994	42,507 ± 5,286	22,591 ± 1,556
10^{-7} M Cortisol	20,346 ± 2,327	15,471 ± 4,964	24,496 ± 4,673	15,900 ± 6,782
10^{-6} M Cortisol	12,389 ± 1,162	7,053 ± 1,024	20,337 ± 3,402	13,339 ± 7,132
10^{-5} M Cortisol	7,359 ± 1,027	8,126 ± 834	10,984 ± 1,610	7,245 ± 1,626
10^{-8} M Epicortisol	78,376 ± 7,297	34,873 ± 9,821	NT[d]	NT
10^{-7} M Epicortisol	64,420 ± 7,257	22,730 ± 3,097	NT	NT
10^{-6} M Epicortisol	52,371 ± 6,503	31,039 ± 2,951	NT	NT
10^{-5} M Epicortisol	52,773 ± 8,543	41,941 ± 4,385	NT	NT

[a] The steroid was added only once—at the time of coincubation with allogeneic lymphocytes.
[b] These cells were incubated with the same amount of ethanol (0.1%) as was present in the 10^{-5} cortisol and epicortisol media.
[c] Mean of triplicate wells ± SEM.
[d] NT = not tested.

at the time of coincubation of skin cells and PBML and maintenance in the medium for 7 days completely inhibited the capacity of lymphocytes to respond to allogeneic skin cells (Table VI).

Effect of Other Agents on the SLR

To determine whether the observed inhibition of the SLR was specific to drugs useful in the treatment of psoriasis, we studied the effect on the SLR of 2 drugs (propranolol and Li_2CO_3) not used for psoriasis therapy, but reported to exacerbate the disease in some instances [13,14]. Propranolol was tested at concentrations ranging from 10^{-5} M to 10^{-6} M and Li_2CO_3 was tested at concentrations ranging from 0.30 mg/ml to 0.03 mg/ml. These levels are in the therapeutic range reached in the serum in vivo [15,16]. Both of these drugs were added to the epidermal cell suspension 2 h before coincubation with allogeneic lymphocytes and maintained in the culture medium for 7 days of incubation to insure maximum exposure of cells to the drugs. At these concentrations, neither drug inhibited the SLR. Moreover, the addition of Li_2CO_3 augmented the SLR of 1 individual (not shown).

A variety of other agents were tested for their effects on the SLR by addition to the culture medium but none had any effect (not shown). These drugs included antibiotics (penicillin 100 units/ml, streptomycin 100 μg/ml, and gentamicin 50 μg/ml) and the anti-inflammatory agent indomethacin (1 μg/ml and 10 μg/ml).

The Effect of Anthralin and Hyperthermia on the Viability of Dispersed Epidermal Cells

To assess the possibility that the inhibition of the SLR was due to a direct lethal effect of anthralin on skin cells, the viability of anthralin-treated cells was compared to that of cells incubated with medium/acetone alone (Table VII). Over a 48-h time period no significant difference in viability between anthralin-pretreated and control cells at all concentrations tested (up to 10 μg/ml anthralin) was seen (Table VII). To determine whether the viability of total skin cells was affected by hyperthermia over a 48-h period after treatment, the viability of hyperthermia-treated cells also was determined using FDA/EB (Table VII). Over 48 h, the viability of total skin cells treated for up to 4.5 h at 43°C did not differ significantly from untreated skin cells.

The Effect of Hyperthermia and Anthralin on the Viability of LC

To determine whether the inhibition of the SLR by treatments useful in psoriasis was due to a selective effect of the treatment on the LC subpopulation, we enriched the skin cell suspension for OKT6-positive (OKT6+) cells with a panning method [9]. We and others have shown recently that using this method 70% or more of attached cells are LC [17,18]. As shown in Table VIII, the OKT6+ cells stimulate vigorously whereas OKT6-negative (OKT6−) cells are capable of very little or no stimulation in the SLR. Furthermore, hyperthermia (4 h at 43°C) treatment of OKT6+ cells totally inhibits the capacity of these cells to stimulate in the SLR. Finally, we examined the viability of all cell fractions after hyperthermia and anthralin treatment. In this, as in previous experiments, the viability of unseparated cells and LC-depleted cells did not decrease significantly after either treatment (Tables IX, X). However, the viability of OKT6+ cell fraction was markedly decreased by both anthralin and hyperthermia treatments. The same pattern of selective cytotoxicity by hyperthermia on LC-enriched skin cells was seen in a second separate experiment.

TABLE VI. *Effect of MTX on the SLR*

Concentration of MTX (μg/ml)[a]	Response in SLR (cpm)[b]	
	Donor A	Donor B
None	29,923 ± 4,036[c]	43,831 ± 5,652
0.2	2,894 ± 474	4,398 ± 219
1.0	2,033 ± 21	3,434 ± 521
5.0	1,360 ± 139	3,495 ± 79
50.0	1,750 ± 305	3,697 ± 674

[a] MTX was added to the incubation medium at the time culture was initiated between skin cells and allogeneic lymphocytes.
[b] The incorporation of ^{3}H-T into responder lymphocytes when incubated alone was 1,397 cpm (Donor A) and 2,528 cpm (Donor B).
[c] Mean of triplicate wells ± SEM.

TABLE VII. *Viability of unfractionated skin cells after exposure to various agents*

Treatment	Viability (% viable)[a]		
	Day 0[b]	Day 1	Day 2
None	86[c]	77	56
Acetone (only)	73	82	66
0.1 μg/ml Anthralin[d]	91	NT[e]	60
0.5 μg/ml Anthralin	86	NT	58
2.0 μg/ml Anthralin	86	80	55
10.0 μg/ml Anthralin	83	81	67
43°C for 1 h	83	79	65
43°C for 2 h	84	88	70
43°C for 3 h	83	73	67
43°C for 4.5 h	78	87	63

[a] Viabilities determined with FDA/EB.
[b] The viability of cells was determined immediately after treatment (day 0) and at 24 h (day 1) and 48 h (day 2) after treatment.
[c] Mean of duplicate counts; variation is < 15%.
[d] Cells were pretreated with the indicated anthralin concentrations for 2 h.
[e] N.T = not tested.

TABLE IX. *Viabilities of different skin cell fractions at 1 h after hyperthermia treatment*

Cell treatment	Viable[a] cells in various skin cell fractions (%)		
	Unseparated	LC-enriched	LC-depleted
None	55[b]	79	35
43°C	48	20	41

[a] Viability was determined by the method of FDA/EB staining.
[b] For each determination a total of 400 cells were counted; SEM < 10% for all values.

TABLE VIII. *Effect of hyperthermia on the capacity of LC-enriched and LC-depleted skin cells to stimulate in the SLR*

Stimulator cell pretreatment	Response of allogeneic lymphocytes[a] to various skin cell fractions[b] (cpm)					
	LC-enriched		LC-depleted		Unseparated	
	Donor A	Donor B	Donor A	Donor B	Donor A	Donor B
None	71,752[c]	66,641	8,201	2,719	42,708	26,669
43°C for 4 h	1,682	1,544	4,907	681	11,212	1,141

[a] PBML from Donor A incubated with x-irradiated (6,000 rad) PBML from Donor A incorporated 665 cpm (autologous MLR) and when incubated with x-irradiated PBML from Donor B incorporated 59,151 cpm. PBML from Donor B incubated with x-irradiated PBML from Donor B incorporated 173 cpm and when incubated with x-irradiated PBML from Donor A incorporated 48,676 cpm.
[b] Initial viability for unseparated cells was 92%, for LC-depleted cells was 64%, and for LC-enriched cells was 70%.
[c] Mean of triplicate wells; the SEM is always less than 20%.

TABLE X. *Viabilities of different skin cell fractions after anthralin treatment*

Cell treatment	Viable[a] cells in various skin cell fractions (%)					
	Unseparated		LC-enriched		LC-depleted	
	24 h[b]	48 h	24 h	48 h	24 h	48 h
None[c]	45 ± 4[d]	30 ± 4	51 ± 4	41 ± 1	43 ± 1	33 ± 4
Anthralin[e]	47 ± 3	37 ± 4	10 ± 3	4 ± 1	35 ± 6	32 ± 1

[a] Viability was determined by the method of FDA/EB staining.
[b] The viabilities were determined at 24 and 48 h after drug incubation.
[c] These cells were treated with the same concentration of acetone (0.2%) as the anthralin-treated cells.
[d] Mean of triplicate values ± SD.
[e] These cells were incubated in 10 μg/ml anthralin for 2 h at 37°C; washed twice, and incubated in complete RPMI medium at 37°C.

DISCUSSION

These results, combined with previous reports, indicate that several agents effective in the treatment of psoriasis—anthralin, heat [19], PUVA [2,5], short-wave UV radiation [20], MTX [21], and cortisol—are inhibitory of the SLR at in vitro concentrations comparable to therapeutic dose levels. By contrast, a variety of antibiotics, epicortisol, and the nonsteroidal anti-inflammatory agent indomethacin as well as 2 agents (Li_2CO_3 and propranolol) thought to exacerbate the disease had no inhibitory effect on the SLR.

We and others have shown that only a small minority of skin cells have the capacity to stimulate in the SLR—those that express HLA-D/DR antigen and which, by distribution and morphology, correspond to bone marrow-derived LC [6,18,22–24]. Thus, it is the functional integrity of these cells that one assays in the SLR. In the series of experiments reported here, we have demonstrated that the antipsoriatic treatments hyperthermia and anthralin act directly on LC. On this basis, it seems likely that the inhibitory effect of both of these agents on the SLR is mediated at least in part by the death of LC. Indeed, a recent report demonstrates that the number of LC in human epidermis decreases as monitored by electron microscopy after PUVA therapy [25]. Furthermore, after PUVA treatment the number of cells expressing HLA-DR in the epidermis is markedly reduced [26]. Accordingly, the principal effects of locally active antipsoriatic agents such as hyperthermia, short-wave UV radiation, and anthralin may be to interfere with immune cell interactions in the skin that lead secondarily to proliferation of keratinocytes. Such a mechanism has been suggested previously to explain the therapeutic effects of PUVA [5,27].

Two drugs, cortisol and MTX, inhibit the SLR only when present continuously during the culture with allogeneic PBML. Furthermore, cortisol inhibits the capacity of PBML to respond to lectin, suggesting that inhibition in the SLR by this drug may be due, at least in part, to an effect on responder cells. Regardless of the precise site of action of cortisol and MTX (responder cells or stimulator cells), the observation that each drug is inhibitory of the SLR clearly demonstrates that these drugs inhibit a cellular immune response.

Experiments by us and others have documented that cortisol does not inhibit proliferation of human keratinocytes cultured in vitro. Indeed, cortisol (1.1×10^{-6} M) has been used to stimulate the growth of cultured human keratinocytes [28]. These observations appear paradoxical in view of the clearly established clinical efficacy of corticosteroids in the treatment of the hyperproliferation of keratinocytes that is characteristic of psoriasis. On the other hand, the concept that the hyperproliferation of keratinocytes seen in the psoriatic plaque is due to an abnormal stimulation of keratinocyte growth by another cell type accommodates both the experimental and clinical observations. On this basis, we propose that immunocompetent cells play a major role in the pathogenesis of psoriasis and that antipsoriatic agents primarily affect immunocompetent cells and only secondarily lead to inhibition of keratinocyte growth. Hopefully, studies of the possible interactions between LC and keratinocytes will clarify the role of the immune system in psoriasis.

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